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## Sir John Cornforth AC CBE FRS: his biosynthetic work

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### ABSTRACT

Sir John Cornforth's work on the stereochemistry of enzyme reactions involved in the biosynthesis of squalene and cholesterol and in the formation and metabolism of a chiral methyl group in acetyl co-enzyme A, is reviewed.

**Keywords:** acetyl co-enzyme A, cholesterol, citric acid, chiral methyl, farnesyl diphosphate, mevalonic acid, squalene

### 1. Introduction

In December 1953, Sir Robert Robinson began his famous Weizmann Memorial lectures on *The structural relations of natural products*<sup>1</sup> with the sentence: "Organic chemists have often been tempted to leave the security of their own proper pastures and to graze, albeit speculatively, in the alternative fields of biochemistry." Sir John Cornforth, in collaboration with biochemists, devised experiments with results that addressed significant aspects of that speculation. In 1947, Sir Robert Robinson had been awarded the Nobel Prize for his investigations on plant products of biological importance and in 1975 his pupil, Sir John Cornforth, was awarded the Nobel Prize for his work on the stereochemistry of enzyme reactions.

There have been a number of stages in the study of the biosynthesis of natural products. The first period, prior to the Second World War, was primarily speculative in which the analysis of the structures and co-occurrence of natural products led to the proposal of common building blocks and structural relationships. The second period, which developed after the Second World War and extended into the 1960s, involved the experimental identification of these building blocks and the sequences which inter-related natural products. The third period, which began in the 1950s and has lasted to the present, has involved the elucidation of the stereochemistry and mechanism of these processes. This has led to the identification and isolation of the enzyme systems that mediate key biosynthetic steps and more recently, to an understanding of the genetics that control these steps. Sir John Cornforth's contributions to our understanding of the stereochemistry of enzyme reactions fall into the second and third stages and were carried out mainly in the period from 1948.

A number of features contributed to the development of this work. In 1948, A.G. Ogston pointed out<sup>2</sup> that when a molecule with the structure Caabc **1** having two identical groups 'a' attached to a carbon atom, binds to the chiral cavity of an enzyme system using a three-point binding of the groups a,b,c, the originally identical groups 'a' are differentiated, *i.e.* the chirality of the enzyme system is reflected in a pro-chirality of the molecule Caabc and in particular of the groups 'a'. At the time this led to an understanding of the distribution of radioactivity in citric acid **2** metabolism. Cornforth not only realised that this had much wider implications in biosynthesis but he was also able to return to the citric acid system later in his career.

The increasing post-war availability of the isotopes of carbon and hydrogen and the techniques for measuring them meant that many biosynthetic experiments which had previously relied on changes in the quantities of metabolite production to demonstrate precursor:metabolite relationships, could be examined with greater confidence<sup>3</sup>. From 1948 at the National Institute for Medical Research, Sir John

Cornforth was able to work with a biochemist, George Popják. This collaboration continued when they moved to the Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent, run by Shell. When Popják went to the University of California, Los Angeles in 1968, Cornforth began a collaboration with Professor Hermann Eggerer, a biochemist at the Institut für Biochemie der Universität München. Whereas Cornforth's research with Popják was mainly on squalene and sterol biosynthesis, his studies with Eggerer involved the chiral methyl group in which two of the hydrogen atoms were stereospecifically replaced by deuterium ( $^2\text{H}$ ) and tritium ( $^3\text{H}$ ).

## 2. The biosynthesis of cholesterol

### 2.1 The background

The sterols, the bile acids and the steroid hormones have many biological roles and consequently an understanding of their biosynthesis was of considerable importance. It has subsequently been shown that a number of major drugs such as the statins affect their biosynthesis. Having been concerned with the total synthesis of cholesterol, it was not surprising that Cornforth turned his attention to sterol biosynthesis. His work involved locating the position of the simple biosynthetic building blocks in the sterols and their precursors and then establishing the stereochemistry and mechanism of the biochemical processes by which they were assembled. This entailed not only the synthesis of the specifically labelled biosynthetic precursors but also a careful analysis of their metabolites to determine the eventual site of the labels.

There were a number of antecedents to Cornforth's work in this area. In 1922, Ruzicka had proposed<sup>4</sup> that terpenoids were assembled by the head-to-tail linkage of  $\text{C}_5$  isoprene units. When the structures of squalene **4** and cholesterol **6** were established, it was clear that they fell into this family of natural products. In 1934, Robinson developing an earlier proposal by Channon that squalene was a precursor of cholesterol, suggested<sup>5</sup> a folding **5** of squalene to generate the carbon skeleton of the sterols. The subsequent elucidation of the structures of the tetracyclic triterpenes such as lanosterol, led R.B. Woodward in 1953 to propose<sup>6</sup> an alternative folding of squalene **7**.

### 2.2 The acetate labelling pattern of cholesterol

Experiments by Rittenberg and Schoenheimer in 1937 and by Bloch and Rittenberg in 1942 had established<sup>7</sup> the incorporation of  $[\text{}^2\text{H}_3]$ -acetate into cholesterol. Degradative experiments reported<sup>8</sup> by Bloch in 1952 identified the labelling pattern of the side chain from the methyl and carboxyl groups of  $[\text{}^{14}\text{C}]$ -acetate. However, establishing the labelling pattern of the tetracyclic carbon skeleton was a more difficult problem. Cornforth with his experience of steroid synthesis, rose to the challenge. Popják carried out the biochemical work by feeding separate samples of, on the one hand  $[\text{}^{14}\text{C}]$ -carboxyl and on the other  $[\text{}^{14}\text{C}]$ -methyl-labelled acetate **3**, to rat liver slices. He was able to obtain a sufficiently high incorporation (2.5–3%) into cholesterol to facilitate Cornforth's chemical studies<sup>9</sup>. Despite the extensive known chemistry of cholesterol, the unambiguous identification of each carbon atom in this part of the molecule required the development of novel strategies<sup>10,11</sup> to dismember the tetracyclic ring system. These involved ozonolysis of alkenes in rings B and D and then the stepwise degradation of the resulting aldehydes and ketones in order to isolate the individual carbon atoms in a form whose radioactivity could be measured. The results of this work established the labelling pattern of cholesterol **6**. Degradation of squalene, again involving ozonolysis, showed<sup>12</sup> that it possessed the labelling pattern as shown in **4**. Taken together, these pieces of work defined the way in which squalene is folded to form cholesterol as **7** (the Woodward scheme, note the origin of the carbon atoms of ring C). Although the cyclisation was originally formulated as involving  $[\text{OH}^+]$ , it is now known to proceed *via* the 2,3-epoxide and it is not completely concerted.

Almost incidental to this work but, nevertheless, of great importance, was the correlation of the degradation product from ring D with (+)-citronellal which served to establish the absolute stereochemistry of cholesterol<sup>13</sup>.

### 2.3 The role of mevalonic acid

The elucidation of the role of mevalonic acid **8** in the biosynthesis of terpenoids and steroids was based on the discovery<sup>14</sup> in 1956 by Tavormina, Gibbs and Huff that the *Lactobacillus casei* growth factor ( $\beta$ -hydroxy- $\beta$ -methyl- $\delta$ -valerolactone, mevalonic acid lactone **9**) was a precursor of cholesterol<sup>15</sup>. Cornforth prepared [2-<sup>14</sup>C]-mevalonic acid lactone by a Reformatski reaction between methyl [2-<sup>14</sup>C]-bromoacetate and 1-acetoxybutan-3-one and, in 1957, he was able to show<sup>16,17</sup> that it was also a precursor of squalene. This paved the way for his important series of experiments on the stereochemistry of squalene, and consequently cholesterol, biosynthesis.

### 2.4 The stereochemistry of squalene biosynthesis

Although the C<sub>30</sub> squalene is a symmetrical molecule, its enzymatic formation from six mevalonic acid units involves a number of stereospecific processes. Various workers such as Lynen in Germany<sup>18</sup> and Bloch in the USA<sup>19</sup> had shown that the C<sub>6</sub> mevalonic acid **8** was converted into a C<sub>5</sub> compound, isopentenyl diphosphate **11**, which formed the isoprene unit from which the terpenes and steroids were constructed. Isopentenyl diphosphate underwent isomerisation to 3,3-dimethylallyl diphosphate **10** and, on combination with further isopentenyl diphosphate units, this formed firstly the C<sub>10</sub> geranyl diphosphate **12** and subsequently the C<sub>15</sub> farnesyl diphosphate **13**, two molecules of which were linked together to form squalene. Cornforth investigated the stereochemistry of these steps in studies which represent the transition from experiments to define the nature of the basic building blocks of sterol biosynthesis, to experiments which were designed to examine the stereochemistry and possible mechanisms by which these building blocks were assembled to form the sterols.

In a logical dissection of squalene biosynthesis from mevalonic acid, Cornforth identified<sup>20</sup> 14 steps at which asymmetric labelling of precursors might uncover stereospecificity in the enzyme reactions. If each of these steps could proceed in one of two stereochemical senses, there would be 2<sup>14</sup> or 16,384 possible routes to be distinguished. These biosynthetic questions included the following:

- When mevalonic acid **8** as its 5-diphosphate is converted into isopentenyl diphosphate **11**, is the elimination of the C-3 hydroxyl group and the carboxyl group a *cis* or a *trans* process?
- When isopentenyl diphosphate **11** is isomerised to 3,3-dimethylallyl diphosphate **10**, to which face of the double bond is the new proton added, and which of the two hydrogens at C-2 is lost and, consequently, is the new methyl group *cis* or *trans* to the C-1 diphosphate?
- In the reaction of 3,3-dimethylallyl diphosphate **10** with isopentenyl diphosphate **11** to form the C<sub>10</sub> geranyl diphosphate **12**, which side of the double bond of the isopentenyl diphosphate **11** is attacked by the 3,3-dimethylallyl diphosphate **10**, and what is the stereochemistry of the hydrogen which is eliminated from C-2 of the isopentenyl diphosphate **11** to create the new alkene in geranyl diphosphate **12**?
- When the new C–C bond is formed in this step, does this take place with inversion or retention of configuration at C-1 of the allylic diphosphate **10**?
- An identical set of questions was formulated for the conversion of geranyl diphosphate **12** to farnesyl diphosphate **13**.

When two molecules of farnesyl diphosphate **13** are combined in a head-to-head manner to form squalene **4**, it was found that one hydrogen from C-1 of one of the farnesyl diphosphate units was

replaced by hydrogen from NADPH. What was the stereochemical origin of this hydrogen on the NADPH and what was its fate, and finally what changes were there to the configuration at C-1 of the other molecule of farnesyl diphosphate **13**?

The investigation of these points required the synthesis of stereospecifically labelled mevalonates<sup>21</sup>.

### 2.5 The synthesis of stereospecifically labelled mevalonates

The use of the stereospecifically deuterated and tritiated mevalonates in the study of sterol biosynthesis, rests on the fact that only the (3*R*) isomer of mevalonic acid **8** acts as a substrate for the first enzymatic step, the mevalonate kinase, *i.e.* the (3*S*)-mevalonate isomer in a racemic mixture is essentially a 'spectator' in the biosynthetic pathway. The strategy for introducing the label at C-4 with a stereospecifically defined relationship to the tertiary alcohol at C-3 depended on the reduction of a 3,4-epoxide by a hydride reagent which proceeded in a *trans* manner. The preparation<sup>22</sup> of the (4*R*) and (4*S*)-[4-<sup>2</sup>H or 4-<sup>3</sup>H] mevalonic acids utilised, in separate experiments, the lactone **14** with a *cis* double bond and the *trans* hydroxy-acid **16**. Their epoxides were reduced with lithium [<sup>2</sup>H or <sup>3</sup>H]-borohydrides to give the labelled mevalonic acids **15** and **17**, respectively, in which there was a *trans* relationship between the hydroxyl group at C-3 and the newly introduced deuterium or tritium isotope at C-4. By interconverting the carboxyl and primary alcohols of mevalonic acid, the chirality of the label which had been introduced at C-4, was transferred to C-2, to generate the (2*S*) and (2*R*)-labelled mevalonates<sup>23</sup>.

Cornforth prepared<sup>24,25</sup> (5-<sup>2</sup>H<sub>2</sub>)- and (3*RS*,5*S*)-[5-<sup>3</sup>H<sub>1</sub>]-mevalonic acids by chemical and chemo-enzymatic methods, respectively. A stereospecific label at C-5 may also be introduced by the enzymatic reduction of mevaldic acid **18** → **19** using mevaldate reductase and labelled NADH or NADPH as coenzyme. [5-<sup>18</sup>O]-Mevalonolactone was prepared by the acid-catalysed hydrolysis of the dimethylacetal of methyl mevaldate with [<sup>18</sup>O]-water followed by reduction with sodium borohydride<sup>26</sup>. A synthesis of (3*R*) and (3*S*)-mevalonolactone from the epimers (+)-linalool and (–)-linalool (**20**) led<sup>27</sup> to a correction of the stereochemical assignment to the latter.

### 2.6 The biosynthesis of farnesyl diphosphate

The fate (**23** → **24**) of these stereospecific labels when the mevalonates were used as substrates for biosynthesis provided many answers to the stereochemical questions which Cornforth had posed. The stereochemistry of the hydrogen atoms at C-2 of isopentenyl diphosphate (corresponding to C-4 of mevalonic acid) that were lost in the formation of 3,3-dimethylallyl diphosphate and in the chain elongation steps, was established<sup>22</sup> by incubating both (4*R*)- and (4*S*)-[4-<sup>2</sup>H]-mevalonates with rat liver slices. Analysis of the resultant farnesyl diphosphate with its *trans* double bonds, showed that only three (4*R*)-deuterium atoms were retained whilst the (4*S*)-deuterium was lost. In contrast, when rubber with its *cis* double bonds is formed, it is the pro-(4*R*)-mevalonoid hydrogen atom which is lost.

The geometry of the (2*R*)- and (2*S*)-[<sup>2</sup>H]-mevalonoid labels in the isopentenol derived from isopentenyl diphosphate showed<sup>23</sup> that there was a *trans* elimination of the carboxyl and the C-3 hydroxyl group of mevalonic acid, in the formation of isopentenyl diphosphate. However, the identification of the face of this alkene which was protonated in the isomerisation to 3,3-dimethylallyl diphosphate, had to wait until the stereochemistry of chiral methyl groups could be elucidated.

The stereochemistry of the carbon–carbon bond formation in the homologation steps was

established<sup>24</sup> by using a sample of (5*R*)-[5-<sup>2</sup>H]-mevalonate to give farnesyl diphosphate. Degradation of this gave [<sup>2</sup>H]-succinic acid **21** in which the methylene groups represent C-1 and C-5 of two separate isoprene units. Cornforth had access to a very sensitive polarimeter with which to measure the small changes in optical rotation with wavelength (optical rotatory dispersion) arising from the chiral replacement of hydrogen by deuterium. Optical rotatory dispersion measurements showed that this was (*R*)-[<sup>2</sup>H]-succinic acid arising from an inversion of configuration at the terminal position of the allylic diphosphate in the formation of the new C–C bonds.

### 2.7 The formation of squalene

The formation of squalene from two molecules of farnesyl diphosphate has turned out to be more complex<sup>28-31</sup> than originally thought. When squalene was biosynthesised by a rat liver enzyme using [5-<sup>2</sup>H<sub>2</sub>]mevalonate as a substrate, eleven out of the possible 12 deuterium atoms were incorporated. One label was lost from the central two carbon atoms of squalene. Ozonolysis of the squalene produced biosynthetically from this mevalonate gave a trideuteriosuccinic acid which was shown to have an (*S*)-configuration. When (*R*)-[1,5,9-<sup>2</sup>H<sub>3</sub>]farnesyl diphosphate, prepared from (5*R*)-[5-<sup>2</sup>H]mevalonate, was used as a substrate, there was no loss of label in the formation of the squalene. Furthermore, ozonolysis of the squalene gave an optically inactive meso (*RS*)-isomer of dideuteriosuccinic acid. Thus there was an overall retention of configuration at C-1 of one molecule of farnesyl diphosphate and an inversion of configuration at the other. The hydrogen that was introduced into the formation of squalene had its origin in NADPH.

Nicotinamide adenine dinucleotide and its phosphate (NAD and NADP) are the co-enzymes for a large number of enzyme-catalysed hydrogen transfer reactions. The hydrogen can be transferred to and from either of the faces of the dihydropyridine ring **22** depending on the particular enzyme. These enzymes were known as ‘A’ or ‘B’ enzymes but the absolute stereochemistry of the hydrogen transfer was unknown. Cornforth prepared<sup>32</sup> enzymatically ‘A’ and ‘B’ samples of NAD<sup>2</sup>H specifically labelled on each of the C-4 hydrogens of the pyridine ring. Degradation of these samples to the corresponding [<sup>2</sup>H]succinic acid and determination of their absolute stereochemistry from their optical rotatory dispersion, showed that the ‘A’ face enzymes transferred the pro-*R* hydrogen and the ‘B’ face enzymes transferred the pro-*S* hydrogen. It was the latter that provided the hydrogen which was introduced into squalene during the linking of the two molecules of farnesyl diphosphate<sup>33</sup>. Subsequent work revealed the intermediacy of pre-squalene phosphate providing an explanation for the replacement of the mevalonoid hydrogen by one from NADPH. A hydrogen atom is lost from one molecule of farnesyl diphosphate in forming the cyclopropane ring of presqualene diphosphate whilst a hydrogen atom is introduced from NADPH during its subsequent conversion to squalene.

### 2.8 The methyl group rearrangement in cholesterol biosynthesis

During the biosynthesis of cholesterol a methyl group (C-18) becomes attached to C-13. The initial cyclisation of squalene would give an intermediate bearing methyl groups at C-8 and C-14. One of these methyls is lost (from C-14) in the formation of cholesterol whilst the other becomes attached to C-13. This could arise by two 1,2-methyl group migrations from C-8 to C-14 and from C-14 to C-13 or by a single 1,3-methyl group rearrangement from C-8 to C-13 leaving the group originally at C-14 to be lost. Cornforth devised<sup>34</sup> an elegant experiment to distinguish between these possibilities. The methyl group rearrangement from C-14 to C-13 takes place within a single isoprene unit and the methyl group becomes attached to a carbon that had originally been C-4 of mevalonate. On the other hand, a 1,3-methyl group rearrangement between C-8 and C-13 takes place between two

separate isoprene units. In order to study this, Cornforth prepared  $[3',4\text{-}^{13}\text{C}_2]$ mevalonic acid **25**. This mevalonate which was suitably diluted with unlabelled mevalonate so that when it was used in a biosynthesis the adjacent isoprene units were not labelled. Degradation of the resultant cholesterol **26** gave  $[^{13}\text{C}_2]$ acetic acid **27** derived from C-13 and C-18, showing that the rearrangement had involved a 1,2-shift within an isoprene unit.

Cornforth's strategy of using stereospecifically labelled mevalonates in squalene and cholesterol biosynthesis provided the stimulus for other workers to carry out numerous experiments to unravel the stereochemistry of the later stages of terpenoid and steroid biosynthesis. Apart from steroid biosynthesis, these have included the stereochemistry of cyclisation, hydroxylation, desaturation and ring-contraction reactions in sesquiterpenoid, diterpenoid and carotenoid biosynthesis.

### 3. The chiral methyl group<sup>35</sup>

#### 3.1 The background

If each of the hydrogen atoms of a methyl group are stereospecifically labelled with the isotopes, protium, deuterium and tritium, the methyl group becomes chiral. The formation and fate of such a methyl group in a biochemical system can shed a light on the stereochemistry of the enzyme reactions that are involved. Thus there are a number of enzyme-mediated reactions in which a methyl group is generated from a methylene. These include the protonation of the methylene of an alkene as in the isomerisation of isopentenyl diphosphate **11** to 3,3-dimethylallyl diphosphate **10**. Another set of enzyme reactions involve the replacement of a leaving group by hydrogen as in the formation of acetyl co-enzyme A by the decomposition of citric acid. In the first set of enzyme reactions there is a stereochemical question concerning the face of an alkene which is protonated by the enzyme to generate the methyl group whilst in the second case the incoming hydrogen from the enzyme can replace the leaving group with retention or inversion of configuration. In each case, if the methylene is stereospecifically labelled with deuterium and tritium, a chiral methyl group may be generated by the addition of protium.

In the reverse situation, a methylene may be generated from a methyl group. For example, a hydrogen of the methyl group of acetyl co-enzyme A may be substituted by another group. The hydrogen may be replaced with retention or inversion of configuration. However, since a methyl group can freely rotate, the discrimination between these processes is based on the primary isotope effect in which replacement of a hydrogen atom will be favoured over a deuterium or tritium. Comparison of the absolute configuration of the deuterium and tritium in the resultant methylene with the chirality of the methyl group in the original acetyl co-enzyme A, will establish the overall stereochemistry of the process.

#### 3.2 The synthesis of the chiral methyl group in acetic acid

Cornforth prepared<sup>35,36</sup> chiral acetic acid containing protium, deuterium and tritium with a defined absolute configuration using reactions of established stereospecificity and a resolution.  $[^2\text{H}]$ -Phenylacetylene **28** was reduced with diimide, which adds hydrogen in a *cis* manner to the alkyne, to give (Z)- $[2\text{-}^2\text{H}]$ -1-phenylethylene **29**. The epoxides (**30**, **31**) of this alkene were then reduced with lithium  $[^3\text{H}]$ -borohydride to give a mixture of (1*R*,2*R*) and (1*S*,2*S*)- $[2\text{-}^2\text{H},2\text{-}^3\text{H}]$ -1-phenylethan-1-ol (**32**, **33**). This racemic mixture was then resolved to give the (1*R*) and (1*S*) enantiomers whose absolute configuration had been established by other studies. The reduction of the epoxide to generate these chiral alcohols related the absolute stereochemistry of the hydrogen isotopes in the methyl group to that of the alcohol. Oxidation of the chiral alcohols to the corresponding ketones followed by a Baeyer–Villiger oxidation gave the chiral acetic acids [**34**(*R*) and **35**(*S*)].

### 3.3 The assay of a chiral methyl group

The method of assay of the chiral acetates depended on the known stereochemistry of two enzyme systems, malate synthase and fumarase. The first enzyme system produces (*S*)-malic acid **37** from acetyl co-enzyme A and glyoxalate **36**, whilst the second enzyme system catalyses (reversibly) the *trans* dehydration of (*S*)-malic acid to fumaric acid. The isotope effect which favours replacement of hydrogen over deuterium from, for example, acetyl co-enzyme A produced from (*R*)-acetic acid **34**, would generate an (*S*)-malic acid with an unequal distribution of the two isotopomers **37** and **38**. *Trans*-dehydration by the fumarase in the first case would lead to a greater retention of tritium than in the second case (**39** and **40** from **35**), thus affording a distinction between the (*R*) and (*S*)-acetates. The assay was applied<sup>37</sup> to the determination of the stereochemistry of protonation of isopentenyl diphosphate **42** to 3,3-dimethylallyl diphosphate **43**. Earlier work had established the geometry of the tritium labels on the double bond of isopentenyl diphosphate when it was formed from (*2R*) and (*2S*)-[2-<sup>3</sup>H]mevalonates **41**. Hence, when these mevalonates were used as substrates with an enzyme system operating in deuterium oxide, the resultant farnesyl diphosphate **44** possessed a chiral methyl group at the start of the chain which had arisen from the 3,3-dimethylallyldiphosphate. The farnesyl diphosphate was degraded and the sample of chiral acetate **45** was subjected to the assay. The results showed that the deuterium had been added to the *re* face of the alkene of isopentenyl diphosphate. This completed Cornforth's stereochemical analysis of the pathway from mevalonate to squalene.

### 3.4 Some applications of the chiral methyl group in biosynthetic experiments

The study of the metabolism of citric acid has played a central role in understanding the biochemical pathways of primary metabolism. Cornforth applied the chiral methyl group to stereochemical investigations of a number of these enzyme reactions<sup>38-40</sup>. Although citric acid is a chemically symmetrical molecule, as Ogston has shown, the acetic acid arms are pro-chiral and, in the presence of enzyme systems such as citrate synthases and lyases, they are distinguishable. The *si*-citrate synthase and lyase catalyse the introduction and removal, respectively, of an acetyl co-enzyme A unit **46** to and from the carbonyl group of oxaloacetic acid **47** operating on one arm of citric acid **48**, whilst the *re*-synthase and ATP-citrate lyase operate on the other arm. Using samples of labelled citric acid which were cleaved by the *si*-citrate lyase in the presence of deuterium oxide to generate acetyl co-enzyme A, Cornforth showed that this enzyme had operated with overall inversion of configuration.

Succinic acid is also formed enzymatically from citric acid. The stereochemistry of the enzymatic dehydrogenation of the succinic acid to fumaric acid was used<sup>41</sup> to establish the stereochemistry of samples of succinic acid obtained from citric acid and, in turn, that of the parent citric acid. By following the fate of the chiral labels in this sequence of enzyme reactions, Cornforth was able to show that the initial condensation between oxaloacetic acid and acetyl co-enzyme A to form citric acid, had also proceeded with inversion of configuration. In the conversion of (*S*)-malic acid to pyruvic acid, Cornforth was able to show<sup>42</sup> that the replacement of the carboxyl group by hydrogen occurred without an overall change of configuration.

Cornforth then turned<sup>43</sup> his attention to the formation of the mevalonic acid precursor, 3-hydroxy-3-methylglutaryl co-enzyme A and to the chain elongation steps (**49** → **52**) of fatty acid biosynthesis<sup>44,45</sup>. For this he used the enantiomers of malonyl co-enzyme A **50** which, in fatty acid biosynthesis, is formed by the carboxylation of acetyl co-enzyme A. The overall results from Cornforth's studies were that the carboxylation of the acetyl co-enzyme A occurred with retention of configuration whilst the condensation step in fatty acid biosynthesis proceeded with inversion of configuration.

Cornforth's work and parallel studies by Arigoni, paved the way for important stereochemical and



mechanistic studies by other workers involving acetyl co-enzyme A and the methyl group in biosynthesis. These included the stereochemistry of the biosynthetic reactions involving acetyl co-enzyme A in polyketide biosynthesis, the methyl transferases from *S*-adenosyl methionine, the methane mono-oxygenases and rearrangements catalysed by the co-enzyme B<sub>12</sub> such as the conversion of 2-methylideneglutarate to 3-methylitaconate<sup>46</sup>. Many of these studies were multi-national and Cornforth was able to make significant contributions to them.

Cornforth's pioneering and perceptive work on biosynthesis and, in particular, on the stereochemistry of enzyme reactions, has made immense contributions to the greater understanding that we have today of the chemistry that occurs within the active site of many key enzymes. Furthermore, the labelled substrates and methodology, which he developed, have been applied by many others and have had a major impact in replacing the speculation described by Robinson in the Weizmann lectures<sup>1</sup>, by experimental fact.

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